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Technical note

A rapid high throughput proteomic method based on profiling of proteolytic free peptides to assess post-delivery degradation of placental tissue

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ABSTRACT

A rapid method to determine quality for placental proteomic studies is required due to varying lengths of time between delivery and sampling in routine protocols. We developed a rapid 10 min LC-MS based scanning method to profile free peptides liberated from natural proteolytic degradation. The assay was applied to placenta samples obtained following refrigeration for varying time periods post-delivery (12 h, +24 h, +48 h and +72 h). Analysis reveals time dependant overlapping profiles for groups <24 to +48 h with greatest variation in the +72 h group, indicating that significant proteolysis affects tissue integrity between 48 and 72 h.

1. Introduction

With increasing interest in maximising use of existing placental sample banks for novel clinical studies, there is a need for a cheap, rapid and reliable method to determine sample integrity over varying periods and conditions of storage [1,2]. The aim of this study was to provide proof of principle data for a rapid, cheap, mass spectrometry based assay which could provide an estimate of the degradation state of the placenta, which would be applicable to selection and interpretation of routinely collected placentas for future proteomic studies.

Modern molecular methods are heavily dependent on quality assurance to support the reliability and validity of experimental data. Biomarker discovery places extra emphasis on the need for robust handling protocols for samples procured for biobanking, but for many existing clinical research studies there is currently no comprehensive high-throughput, automated assessment available for overall protein quality [3]. Protein concentration can be estimated using a protein assay (BSA, Lowry assay, Bradford assay). If the estimated yield is non-proportional to the amount of sample used, proteins are assumed to be degraded, or absent. SDS-PAGE gel electrophoresis can also be crudely used to estimate protein integrity, however, unlike nucleic acids, which have an unequivocal UV absorbance spectra and molecular weight, since proteins are variable in size, charge and hydrophobicity, results are less reproducible.

The issue of assessing sample integrity is of particular importance for studies of placental tissue since variation in time between birth, transport to the laboratory, tissue processing and storage can affect sample integrity. In clinical contexts the interval between birth and sampling may be as much as 12 h, however for research studies assessing transcriptome and RNA much shorter intervals (under 45 min) are recommended [4]. These require specialist protocols and are expensive in terms of personnel and reagents required to stabilise the tissue. Hence in routine clinical practice or large scale multicentre biobanking studies where the diagnostic or processing laboratory may be distant from the delivery suite sampling interval may be up to several days, especially if initial refrigeration and triage is performed [5].

An investigation into the effects of prolonged storage interval (over 12 h after birth) will provide novel data on the effects of an

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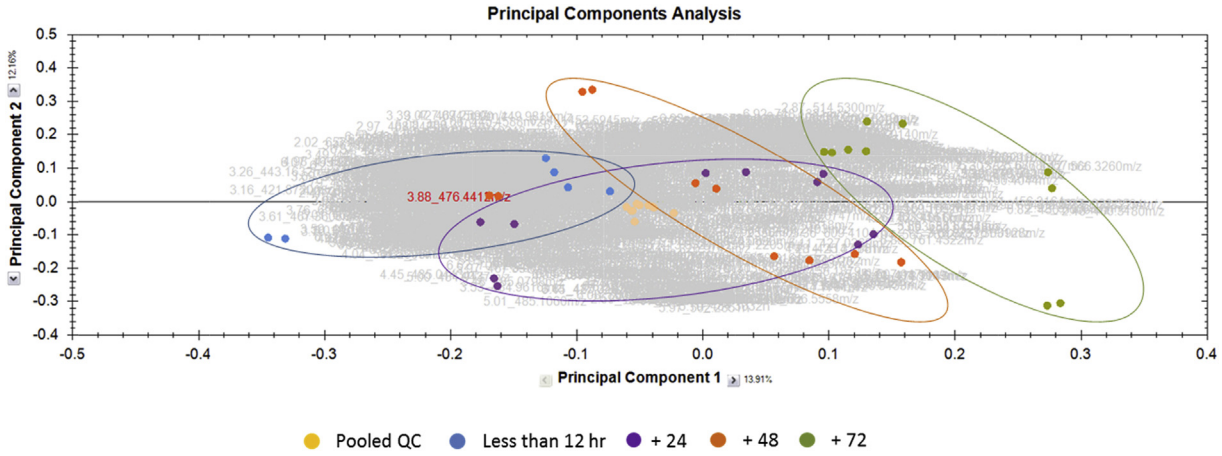

Fig. 1. PCA plot of placental free peptide analysis time points. The cluster regions of each time point are encircled. The <24 h group segregates completely from +48 h to +72 h group but the <24 h period overlaps between <24 and + 48 h. The most distinct group is the +72 h group, which segregates from other groups.

extended storage interval outside the range of artificially controlled scientific experiments, thereby influencing which placental samples from more general collections might be useful for biomarker studies. These data are particularly useful for large scale birth cohort studies which may not analyse samples for some considerable time and where effects of sample handling and varying periods of storage to guide sample collection, inclusion criteria and downstream assay selection.

The monitoring of proteolytic degradation of intact structural proteins has recently been proposed as a way of estimating time of death [6,7]. A change in the proteome due to natural proteolysis will also reflect in the 'peptidome'. Therefore we have developed a rapid and simple approach based on the methods typically used in metabolic profiling [8,9] that can examine overall changes in the peptidome that occur as a result of naturally occurring proteolytic degradation.

2. Methods

Detailed methods are provided in Supplementary Data. Briefly the method involves the solubilisation of frozen placental tissue samples, standardised to total protein content. Free peptides liberated from the tissue that are potential proteolytic degradation products, are extracted and analysed using a triple quadrupole mass spectrometer [10]. The data were analysed using pattern profiling principle component analysis (PCA), which identifies the similarity of the free peptide profiles between samples. To test the method we analysed placentas that had known intervals of <12, 24, 48 and 72 h from delivery to sampling and −80 °C storage.

3. Results & discussion

Fig. 1 shows the PCA plot of 871 detected masses that were doubly charged and not present in the tissue blank. Pooled QC runs are clustered tightly in the middle of the plot, indicating the MS scanning performed well. Overall, there is a gradual overlapping of profiles between time points, whereas the +72 h group appears to cluster significantly separately from the other groups, indicating that it is between 48 and 72 h that the greatest change to the free peptide composition occurs. The +48 h points segregate from those <24 h and +72 h, but with overlap with the +24 h group. These data suggest that there is a point between +24 and + 48 h where the free peptide composition begins to change significantly, with more marked differences by +72 h. Further analysis demonstrates that the free peptide composition is complex and consists of different peptides, some of which decrease (n = 167) and some

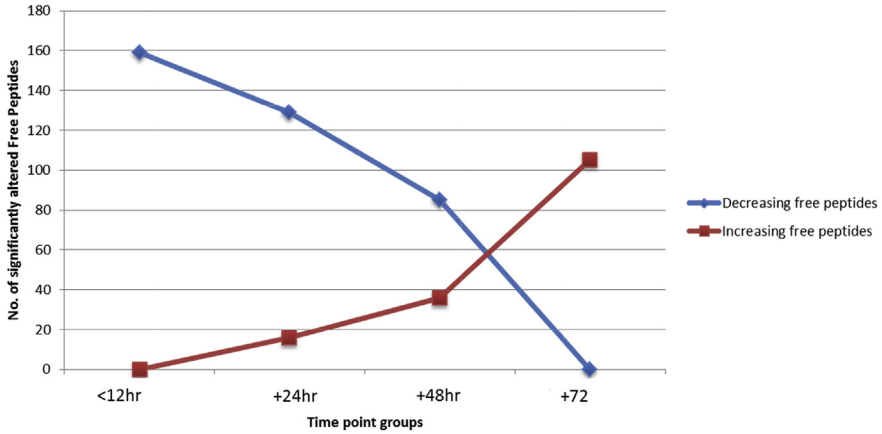

Fig. 2. Trending peptides significantly up regulated ($p < 0.05$) in either the <12 h or +72 h group compared with all other time points were measured along the time point groups and were seen to decrease over time (Fig. 2) with the greatest observed decrease between +48 and +72 h. The greatest increase of peptides is again between the +48 and + 72 h groups. The peptides that increase over time do not appear to be degradation products of the decreasing peptides as the average *m/z* (758.96 ± 211) of the increasing peptides is significantly larger ($p < 0.0001$) than those peptides that decrease (617.69 ± 160). These increasing peptides are more likely to originate from proteolysis of larger proteins.

increase over time (n = 55) compared with the first <24 h group (Fig. 2); these are likely representing both end products and intermediate products of degradation. Free peptide *m*/*z* values used in this analysis are given as Supplementary Data.

This rapid LC-MS scanning assay shows that the free peptide profiling of placental tissue samples can be used to reflect the integrity of the tissue for subsequent proteomic analysis. The assay is simple to perform and does not rely on the analysis or identification of individual proteins, removing the need for antibody methods. With further development this approach may also have potential application for other uses such as determining the timing and mechanisms of intrauterine deaths and impact of storage duration on sample quality.

Disclosure statement

The authors report no conflict of interest.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.placenta.2016.04.014.

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